



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 14226-12PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/CA 03/01477	International filing date (day/month/year) 25.09.2003	Priority date (day/month/year) 26.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/12		
Applicant CENTRE FOR TRANSLATIONAL RESEARCH IN CANCER et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 5 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p>		
Date of submission of the demand 23.04.2004	Date of completion of this report 11.01.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Gurdjian, D Telephone No. +31 70 340-3388 	

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/CA 03/01477**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-39 as originally filed

Claims, Numbers

1-37 filed with telefax on 29.10.2004

Drawings, Sheets

1/12-12/12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☒ furnished subsequently to this Authority in written form.
☒ furnished subsequently to this Authority in computer readable form.
☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 19-24 as far as concerning the industrial application

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-37
	No: Claims	
Inventive step (IS)	Yes: Claims	1-37
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-18,25-37
	No: Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

- D1: VO N ET AL: "Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP." MOLECULAR AND CELLULAR BIOLOGY. UNITED STATES SEP 2001, vol. 21, no. 18, September 2001 (2001-09), pages 6181-6188, XP002269408 ISSN: 0270-7306
- D2: HÖRLEIN A J ET AL: "Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor." NATURE. ENGLAND 5 OCT 1995, vol. 377, no. 6548, 5 October 1995 (1995-10-05), pages 397-404, XP002269409 ISSN: 0028-0836
- D3: DATABASE TREMBL [Online] 1 December 2001 (2001-12-01), NAGASE, T. ET AL.: "Hypothetical protein KIAA1795 (Fragment)" XP002269412 retrieved from EBI Database accession no. Q96JN0

The present application relates to the LCoR transcriptional corepressor, having the molecular sequence data with seq.1,2 from fig.1D, that is binding to the nuclear receptor estrogen receptor through a single LXXLL motif at positions 53-57 and is binding to the C-terminal binding protein corepressors CTBP through the motifs PLDLDLTVR at positions 64-70 and VLDELSTK at positions 82-88. A mutant disrupted in the LXXLL shows a disrupted hormone dependent interaction.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19-24 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claims 19-24 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty(Article 33.2 PCT)

D1 discloses that CtBP (carboxyl-terminal binding protein) participates in regulating cellular development and differentiation by associating with a diverse array of *transcriptional repressors*. Most of these interactions occur through a consensus CtBP-binding motif, PXDLS, in the repressor proteins. CtBP was shown to interact with co-repressor RIP140 in vitro and in vivo through a sequence, PIDLSCK, in the amino-terminal third of the RIP140 protein. RIP140 contains nine *LXXLL motifs*. Yeast two-hybrid CtBP interaction assays identified the binding motifs *pldltr* and *vidlstk* from unknown proteins. It discloses that Myt1 and RIZ contain two CtBP-binding motifs. The unacetylated nuclear hormone receptor-interacting protein RIP140 acts as a transcriptional repressor through its interaction with CtBP. RIP140 represses nuclear hormone receptor-dependent transcription, via the estrogen receptor. (see the abstract, table 1, page 6186 left hand column second paragraph and page 6187 right hand column second paragraph)

D2 discloses the nuclear receptor co-repressor NCoR comprising LXXLL at amino acid positions 674-678. The transcription corepression acts via the Receptors of Retinoic Acid or Thyroid Hormone (see the abstract and figure 2)

In view of D1-D2, the subject matter of claims 1-37 covering nuclear receptor co-repressor having LXXLL in its amino acid sequence, and encoded by the nucleotide sequence from fig.1d is new.

2. Inventive step(Article 33.3 PCT)

D3 discloses the Hypothetical protein KIAA1795 showing 100.000% identity (100.000% ungapped) in 433 aa overlap (1-433:140-572) with seq.2 of the present application. It contains a DNA binding, a regulation of transcription, DNA-dependent and a Homeodomain_like domain, however it *was not annotated as a transcription repressor and fails to comprise the LXXL, pldltr and vidlstk motifs* (see the whole document)

D1 is considered to be the closest prior art.

The subject matter of the present application differs from D1 by the provision of the molecular sequence data from fig.1D of the present application .

The problem to be solved is the provision of a nuclear receptor transcriptional with an alternative overall molecular data sequence .

The person skilled in the art would have had the incentive to solve this problem in view of the therapeutical importances of nuclear receptor transcriptional corepressors .

While the prior art is not suggesting any homologous sequence to fig1d possessing LXXL , pldltvr and vldlstk motifs and is not suggesting that any homologous sequence to fig1d would act as transcriptional repressor , the person skilled in the art would have NO reasonable expectation of succes of cloning the nuclear receptor transcriptional corepressor with the molecular sequence data of fig.1d of the present application . The subject matter of claims 1-37 is hence inventive .

-3. 11. 2004

WHAT IS CLAIMED IS:

(55)

1. An isolated corepressor polypeptide encoded by the nucleotide sequence as set forth in Fig. 1D and having an amino acid sequence which comprises at least one LXXLL nuclear receptor interacting NR box motif wherein L is leucine and X is any amino acid residue, said polypeptide operably interactable with a nuclear receptor to actively repress transcription of DNA.
2. The isolated polypeptide of claim 1, wherein said polypeptide is operably interactable with a nuclear receptor in one of a ligand-dependent and partially ligand-dependent manner.
3. The isolated polypeptide of claim 2, wherein the nuclear receptor comprises a class I or a class II nuclear receptor.
4. The isolated polypeptide of claim 3, wherein the nuclear receptor is selected from the group consisting of ER α , ER β , GR, PR, VDR, RAR α , RAR β , RAR γ and RXR α .
5. An isolated corepressor polypeptide essentially having an amino acid sequence as set forth at Fig. 1D comprising at least one modification of the amino acid sequence.
6. The isolated polypeptide of claim 5, wherein said modification comprises at least one point mutation in the region of the sequence from nucleotides 53 to 57.
7. The isolated polypeptide of claim 6, wherein the sequence from nucleotides 53 to 57 comprises the sequence LSKAA.

8. An isolated corepressor polypeptide encoded by the nucleotide sequence as set forth in Fig. 1D and having within its amino acid sequence at least two C-terminal binding protein interaction motifs, said first C-terminal binding protein interaction motif comprising the sequence PLDLTVR, and said second C-terminal binding protein interaction motif comprising the sequence VLDLSTK, said polypeptide operably interactable with a C-terminal binding protein (CtBP) corepressor in a pathway to repress expression of DNA.

9. The isolated polypeptide of claim 8, wherein the CtBP corepressor is selected from the group consisting of CtBP1 and CtBP2.

10. The isolated polypeptide of claim 8 comprising the amino acid sequence as set forth in Fig. 1D.

11. An isolated polynucleotide coding for the polypeptide of claim 5.

12. An expression vector comprising the polynucleotide of claim 11 operably linked to a promoter for expression in a host cell.

13. A host cell stably transformed with the expression vector of claim 12.

14. An antibody that specifically binds to the polypeptide of claim 1.

15. An antibody that specifically binds to the polypeptide of claim 5.

16. An antibody that specifically binds to the polypeptide of claim 8.

17. A transgenic knock-out mouse comprising disruption in an endogenous gene which encodes for a corepressor polypeptide having a sequence as set forth in Fig. 1D, wherein said disruption has been introduced into its genome by a recombinant DNA construct stably integrated into the genome of said mouse or an ancestor thereof, wherein the disruption of the corepressor gene reduces expression of said corepressor causing altered active transcription of DNA associated with the corepressor.

18. The transgenic knock-out mouse of claim 17, wherein the altered active transcription of DNA is increased relative to wild type.

19. A method of modulating a cell comprising a gene which encodes for a corepressor polypeptide having a sequence as set forth in Fig. 1D, said method comprising the steps of introducing into said cell the isolated polynucleotide according to claim 5, whereby expression of the corepressor polypeptide is modulated.

20. A method of inhibiting ligand-dependent transactivation in a cell by one of a class I and class II nuclear receptor comprising subjecting said cell to a corepressor amount of the polypeptide of claim 1.

21. The method of claim 20, wherein the nuclear receptor is selected from the group consisting of $ER\alpha$, $ER\beta$, GR, PR, VDR, $RAR\alpha$, $RAR\beta$, and $RAR\gamma$.

22. A method of repressing nuclear-receptor mediated transcription in a cell comprising providing a ligand-dependent corepressor amount of the polypeptide of claim 1 to said cell.

23. A method of modulating steroid hormone signaling in a cell comprising providing a ligand-dependent corepressor amount of the polypeptide of claim 1 to said cell.

24. A method of regulating gene expression in a cell comprising providing the polypeptide as set forth at claim 8, wherein the polypeptide is operable to interact with at least one protein in a pathway to regulate gene expression.

25. The method of claim 24, wherein the protein comprises a C-terminal binding protein corepressor.

26. The method of claim 25 wherein the C-terminal binding protein corepressor is selected from the group consisting of CtBP-1 and CtBP-2.

27. Use of the corepressor polypeptide of claim 1 to inhibit ligand-dependent transactivation in a cell by one of a class I and class II nuclear receptor.

28. The use of claim 27, wherein the nuclear receptor is selected from the group consisting of ER α , ER β , VDR, RAR α , RAR β , and RAR γ .

29. Use of the corepressor polypeptide of claim 1 to repress nuclear-receptor mediated transcription in a cell.

30. Use of the polypeptide of claim 1 to modulate steroid hormone signaling in a cell.

31. Use of the polypeptide of claim 8 to regulate gene expression in a cell.

32. Use of the polypeptide of claim 1 in an assay to select, for therapeutic purposes, compounds that modulate transcription of gene expression associated with the polypeptide.

33. Use of the polypeptide of claim 8 in an assay to select, for therapeutic purposes, compounds that modulate transcription of gene expression associated with the polypeptide.

34. A method for assaying for compounds capable of modulating the activity of a corepressor polypeptide of claim 1 or an active variant thereof to actively modify transcription of DNA comprising the steps of:

- (a) providing a corepressor polypeptide of claim 1 or an active variant thereof;
- (b) contacting the corepressor polypeptide with a nuclear receptor in the presence and absence of the compound; and
- (c) measuring the modulation in activity of repression of DNA translation of the corepressor polypeptide.

35. A method for assaying for compounds capable of affording selective recruitment of the corepressor polypeptide of claim 1 in the presence of a ligand of a nuclear receptor, wherein the corepressor is operably interactable with the nuclear receptor to actively repress transcription of DNA in the presence of the ligand.

36. The method of claim 35, wherein the ligand comprises estrogen or an estrogen-like compound and the repressed DNA transcription products are implicated in hormone-dependent cancer.

37. The method of claim 36, wherein the hormone-dependent cancer is selected from the group consisting of hormone-dependent breast cancer and hormone-dependent uterine cancer.